

Claims

1. A method for detecting at least one target polynucleotide sequence in a sample, the
5 method comprising:

forming a ligation mixture comprising the sample and a plurality of different ligation
probe sets, each ligation probe set comprising (a) at least one first ligation probe comprising
a first target-specific portion, and (b) at least one second ligation probe comprising a second
target-specific portion, wherein the first or second probes in each set further comprise an
10 identifier tag portion that identifies the probe that contains the identifier tag portion, and
wherein the first and second target-specific portions of the first and second ligation probes
from at least one ligation probe set are hybridized to adjacent sequences on a target
polynucleotide;

subjecting the ligation reaction mixture to at least one cycle of ligation, thereby
15 forming at least one first strand comprising the first target-specific portion, the second target-
specific portion, and the identifier tag portion of at least one ligation probe set;

forming one or more complexes, wherein each complex comprises a first strand
hybridized to a mobility probe, said mobility probe comprising (a) a mobility defining moiety
that imparts an identifying mobility to the mobility probe, and (b) a tag portion complement,
20 wherein the tag portion complement is hybridized to the complementary tag portion on the
first strand in one or more complexes;

releasing one or more different mobility probes from the one or more complexes, and
detecting the one or more released mobility probes using a mobility-dependent
analysis technique (MDAT).

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2. The method of claim 1 wherein at least one ligation probe set comprises at least
two different first probes having target-specific portions that differ from each other by at
least one nucleotide.

3. The method of claim 1, wherein the first and second probes in at least one ligation probe set hybridize to nearly adjacent complementary target sequences that are separated by a gap of at least one nucleotide, wherein the ligation mixture further comprises a polymerase and free nucleotides, whereby said is filled in prior to ligation.

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4. The method of claim 1, wherein the second ligation probe comprises a 3' primer-specific portion, and an amplification is performed comprising polymerase-mediated extension of a reverse primer, wherein the reverse primer is complementary to the 3' primer-specific portion, to form a linearly amplified strand, wherein the tag portion of the mobility probe is hybridized to a tag portion complement in the linearly amplified strand.

5. The method of claim 1, wherein the first ligation probe comprises a 5' primer-specific portion and the second ligation probe comprises a 3' primer-specific portion, and an amplification reaction is performed comprising polymerase-mediated extension of a forward primer and a reverse primer, wherein the forward primer is complementary to the complement of said 5' primer-specific portion and the reverse primer is complementary to the 3' primer-specific portion, thereby forming exponentially amplified first strand and second strand.

6. The method of claim 5, wherein the plurality of different probe sets comprises at least one probe set comprising (i) first probes having a universal 5' forward primer-specific portion, and (ii) second probes having a universal 3' reverse primer-specific portion, and said amplifying comprises amplifying first and second strands of the at least one probe sets using a single universal primer set comprising a universal forward primer and a universal reverse primer.

7. The method according to claim 1, wherein the at least one first probe or the at least one second probe, or both, further comprise an affinity moiety, said method further comprising;

contacting the first strand with a binding moiety,
immobilizing the first strands,
removing un-immobilized reaction components,
hybridizing at least one mobility probe to the remaining immobilized strands,
5 removing unhybridized mobility probes,
eluting the bound mobility probes, and,
determining the identity of the at least one target polynucleotide sequence by the
identity of the mobility probe in a mobility dependent analysis technique.

10 8. The method according to claim 7 wherein the affinity moiety is biotin.

9. The method according to claim 1 further comprising,
incorporating an affinity moiety into the at least one target polynucleotide sequence,
immobilizing the at least one target polynucleotide sequence with a binding moiety specific
15 for the affinity moiety,
hybridizing the at least one immobilized target polynucleotide sequence with the one
or more probe sets, and,
removing unhybridized probes prior to the ligation reaction.

20 10. The method according to claim 1 wherein the 5' portion of the upstream ligation
probe and/or the 3' portion of the downstream ligation probe are resistant to nuclease
digestion, wherein unligated probes are removed with 3' acting and/or 5'-acting
exonucleases.

25 11. The method according to claim 1 wherein the mobility probes further comprise a
label.

12. The method according to claim 11 wherein the label further comprises a fluorophore.

13. The method according to claim 1 wherein the mobility modifier of the mobility probe is chosen from at least one of the following; polyethylene oxide, polyglycolic acid, polylactic acid, polypeptide, oligosaccharide, and polyurethane, polyamide, polysulfonamide, polysulfoxide, and block copolymers thereof, including polymers composed of units of multiple subunits linked by charged or uncharged linking groups.

14. The method according to claim 1 wherein the mobility dependent analysis technique further comprises capillary electrophoresis.

15. The method according to claim 1 wherein the mobility dependent analysis technique further comprises mass spectrometry.

16. A method for detecting at least one target polynucleotide sequence in a sample, the method comprising:

forming a ligation mixture comprising the sample and a plurality of different ligation probe sets, each ligation probe set comprising (a) at least one first ligation probe comprising a first target-specific portion, and (b) at least one second ligation probe comprising a second target-specific portion, wherein the first or second probe in each set further comprises an identifier tag portion that identifies the probe that contains the identifier tag portion, and wherein first and second target-specific portions of first and second ligation probes from at least one ligation probe set are hybridized to adjacent sequences in a target polynucleotide;

subjecting the ligation reaction mixture to at least one cycle of ligation, thereby forming at least one first strand comprising the first target-specific portion, the second target-specific portion, and the identifier tag portion of at least one ligation probe set;

forming a second strand that is complementary to at least one first strand and that comprises sequence portions that are complementary to the first target-specific portion, the second target-specific portion, and the identifier tag of at least one ligation probe set;

amplifying the first strand and the second strand by a PCR,

forming one or more complexes, wherein each complex comprises a first strand or a second strand and a mobility probe, said mobility probe comprising (a) a mobility defining moiety that imparts an identifying mobility or total mass to the mobility probe, and (b) a tag portion or tag portion complement, wherein the tag portion or tag portion complement is hybridized to the complementary tag portion complement or tag portion, respectively, in the first or second strand in one or more complexes;

releasing one or more different mobility probes from the one or more complexes, and, detecting the one or more released mobility probes using a mobility-dependent analysis technique (MDAT).

17. The method of claim 16 wherein at least one ligation probe set comprises at least two different first probes having target-specific portions that differ from each other by a single nucleotide base.

18. The method of claim 16, wherein the first and second probes in at least one ligation probe set hybridize to nearly adjacent complementary target sequences that are separated by a gap of at least one nucleotide, wherein the ligation mixture further comprises a polymerase and free nucleotides, whereby said gap can be filled in prior to ligation.

19. The method of claim 16, wherein the first ligation probe comprises a 5' primer-specific portion and the second ligation probe comprises a 3' primer-specific portion, and the amplification reaction is performed comprising polymerase-mediated extension of a forward primer and a reverse primer, wherein the forward primer is complementary to the complement of said 5' primer-specific portion and the reverse primer is complementary to the 3' primer-specific portion, to form exponentially amplified first strand and second strand.

20. The method of claim 16, wherein the plurality of different probe sets comprises at least one probe set comprising (i) first probes having the same 5' forward primer-specific

portion, and (ii) second probes having the same 3' reverse primer-specific portion, and said amplifying comprises amplifying first and second strands of the at least one probe sets using a single universal primer set comprising a universal forward primer and a universal reverse primer.

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21. The method according to claim 20, wherein the 5' end of the at least one universal reverse primer comprises an affinity moiety, said method further comprising

contacting the amplified first and second strands with a binding moiety,

immobilizing the amplified strands,

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removing un-immobilized reaction components,

eluting and removing the strand lacking the affinity moiety,

hybridizing at least one mobility probe to the remaining immobilized strands,

removing unhybridized mobility probes,

eluting the bound mobility probes, and,

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determining the identity of the at least one target polynucleotide sequence by the identity of the mobility probe in a mobility dependent analysis technique.

22. The method according to claim 21 wherein the affinity moiety is biotin.

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23. The method according to claim 16 further comprising,

incorporating an affinity moiety into the at least one target polynucleotide sequence,

immobilizing the at least one target polynucleotide sequence with a binding moiety specific for the affinity moiety,

hybridizing the at least one immobilized target polynucleotide sequence with the one

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or more probe sets, and,

removing unhybridized probes prior to the ligation reaction.

24. The method according to claim 16 wherein the 5' portion of the upstream ligation probe and/or the 3' portion of the downstream ligation probe are resistant to nuclease digestion, wherein unligated probes are removed with 3' acting and/or 5'-acting
5 exonucleases.

25. The method according to claim 16 wherein the mobility probes further comprises a label.

10 26. The method according to claim 25 wherein the label further comprises a fluorophore.

27. The method according to claim 16 wherein the mobility modifier of the mobility probe is chosen from at least one of the following, polyethylene oxide, polyglycolic acid, polylactic acid, polypeptide, oligosaccharide, and polyurethane, polyamide, polysulfonamide,
15 polysulfoxide, and block copolymers thereof, including polymers composed of units of multiple subunits linked by charged or uncharged linking groups.

28. The method according to claim 16 wherein the mobility dependent analysis technique further comprises capillary electrophoresis.

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29. The method according to claim 16 wherein the mobility dependent analysis technique further comprises capillary electrophoresis.